

- <110> Visible Genetics Inc. Shipman, Robert
- <120> Method and Kit for the Characterization of Antibiotic-Resistance Mutations in Mycobacterium tuberculosis
- <130> VGEN.P-055-WO
- <140>
- <141>
- <150> 60/111,794
- <151> 1998-12-11
- <160> 50
- <170> PatentIn Ver. 2.1
- <210>1
- <211>20
- <212> DNA
- <213> Mycobacterium tuberculosis
- <220>
- <223> rpoB-F amplification primer
- <400>1

tacggtcggc gagctgatcc

20

- <210>2
- <211>20
- <212> DNA
- <213> Mycobacterium tuberculosis
- <220>
- <223> rpoB-R amplification primer
- <400>2

tacggcgttt cgatgaaccc

<210>3

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> rpoB-5s sequencing primer

<400>3

tacggtcggc gagctgatcc

20

<210>4

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> rpoB-3s sequencing primer

<400>4

tacggcgttt cgatgaaccc

20

<210>5

<211>480

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> rpoB (rifampin resistance)

<400> 5

aaaccgacga catcgaccac ttcggcaacc gccgcctgcg tacggtcggc gagctgatcc 60 aaaaccagat ccgggtcggc atgtcgcgga tggagcggt ggtccgggag cggatgacca 120 cccaggacgt ggaggcgatc acaccgcaga cgttgatcaa catccggccg gtggtcgccg 180 cgatcaagga gttcttcggc accagccagc tgagccaatt catggaccag aacaacccgc 240 tgtcggggtt gacccacaag cgccgactgt cggcgctggg gcccggcggt ctgtcacgtg 300 agcgtgccgg getggaggtc cgcgacgtgc acccgtcgca ctacggccgg atgtgcccga 360 tcgaaacccc tgaggggccc aacatcggtc tgatcggct gctgtcggtg tacgcgcgg 420 tcaacccgtt cgggttcatc gaaacgccgt accgcaaggt ggtcgacggc gtggttagcg 480

<210>6

<211>20

<212> DNA <213> Mycobacterium tuberculosis	
<220> <223> katG-F amplification primer	
<400> 6 atggggctga tctacgtgaa	20
<210> 7 <211> 20 <212> DNA <213> Mycobacterium tuberculosis	
<220> <223> katG-R amplification primer	
<400> 7 ggtgttccag ccagcgacgc	20
<210> 8 <211> 20 <212> DNA <213> Mycobacterium tuberculosis	
<220> <223> katG-5s sequencing primer	
<400> 8 atggggctga tctacgtgaa	20
<210> 9 <211> 20 <212> DNA <213> Mycobacterium tuberculosis	
<220> <223> katG-3s sequencing primer	
<400> 9 ggtgttccag ccagcgacgc	20

•

```
<210>10
```

<213> Mycobacterium tuberculosis

<220>

<223> katG (isoniazid resistance)

<400> 10

geteggegat gagegttaca geggtaageg ggatetggag aaccegetgg cegeggtgca 60 gatggggetg atetacgtga acceggaggg geegaaegge aacceggace ceatggeege 120 ggeggtegac attegegaga egttteggeg catggeeatg aacgaegteg aaacagegge 180 getgategte ggeggteaca ettteggtaa gaceeatgge geeggeeegg cegatetggt 240 eggeecegaa ecegaggetg eteegetgga geagatggge ttgggetgga agagetegta 300 tggeacegga aceggtaagg aegegateae eageggeate gaggtegtat ggaegaaeae 360 eeegacgaaa tgggacaaca gttteetega gateetgtae ggetaegagt gggagetgae 420 gaagageeet getggegett ggeaatacae egeeaaggae ggegeeggtg eeggeaeeat 480 eeeggaeeeg tteggegge eagggegete eeggaaeat etggeeaetg aceteteget 540 gegggtggat eegatetatg ageggateae gegtegetgg etggaaeaee eegaggaatt 600 ggeegaegag ttegeeaagg eetggtacaa getgateae egagaeatgg gteeegttge 660

<210>11

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> PR-F amplification primer

<400>11

accactgett tgccgccacc

20

<210> 12

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> PR-R amplification primer

<400> 12

ccgatgagag cggtgagctg

<211>660

<210>13

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> PR-5s sequencing primer

<400> 13

accactgett tgccgccacc

20

<210> 14

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> PR-3s sequencing primer

<400> 14

ccgatgagag cggtgagctg

20

<210> 15

<211>420

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> oxyR-ahpC intergenic region (PR)

<400> 15

atgecetggg ggtgeacega gaceggette egaceacege tegeegeaac gtegaetgge 60 teatategag aatgettgeg geaetgetga accaetgett tgeegeeace geggegaaeg 120 egegaagece ggeeacggee ggetageace tettggegge gatgeegata aatatggtgt 180 gatatateae etttgeetga eagegaette aeggeaegat ggaatgtege aaccaaatge 240 attgteeget ttgatgatga ggagagteat geeaetgeta accattggeg ateaatteee 300 egeetaeeag eteaeegete teateggegg tgaeetgtee aaggtegaeg ceaageagee 360 eggegaetae tteaecacta teaecagtga egaacaceca ggeaagtgge gggtggtgtt 420

<210> 16

<211>20

<212> DNA

<213> Mycobacterium tuberculosis	
<220> <223> fabG-F amplification primer	
<400> 16 cctcgctgcc cagaaaggga	20
<210> 17 <211> 20 <212> DNA <213> Mycobacterium tuberculosis	
<220> <223> fabG-R amplification primer	
<400> 17 atccccggt ttcctccggt	20
<210> 18 <211> 20 <212> DNA <213> Mycobacterium tuberculosis	
<220> <223> fabG-5s sequencing primer	
<400> 18 cctcgctgcc cagaaaggga	20
<210> 19 <211> 20 <212> DNA <213> Mycobacterium tuberculosis	
<220> <223> fabG-3s sequencing primer	
<400> 19 atccccggt ttcctccggt	20

```
<210>20
<211>360
<212> DNA
<213> Mycobacterium tuberculosis
<220>
<223> fabG (isoniazid resistance)
<400>20
agegegacat acetgetgeg caattegtag ggegteaata caceegeage cagggeeteg 60
ctgcccagaa agggatccgt catggtcgaa gtgtgctgag tcacaccgac aaacgtcacg 120
agegtaacce cagtgegaaa gtteeegeeg gaaategeag eeaegttaeg etegtggaca 180
taccgatttc ggcccggccg cggcgagacg ataggttgtc ggggtgactg ccacagccac 240
tgaagggcc aaacccccat tcgtatcccg ttcagtcctg gttaccggag gaaaccgggg 300
gateggetg gegategeae ageggetgge tgeegaegge caeaaggtgg eegteaceea 360
<210>21
<211>20
```

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> s12-F amplification primer

<400>21

cggtagatgc caaccatcca

20

<210> 22

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> s12-R amplification primer

<400> 22

geateageee tteteettet

20

<210> 23

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> s12-5s sequencing primer

<400> 23

cggtagatgc caaccatcca

20

<210> 24

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> s12-3s sequencing primer

<400> 24

gcatcagccc ttctccttct

20

<210> 25

<211>420

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> rpsL/s12 (streptomycin resistance)

<400> 25

cggtagatgc caaccatcca gcagctggtc cgcaagggtc gtcgggacaa gatcagtaag 60 gtcaagaccg cggctctgaa gggcagcccg cagcgtcgtg gtgtatgcac ccgcgtgtac 120 accaccactc cgaagaagcc gaactcggcg cttcggaagg ttgcccgcgt gaagttgacg 180 agtcaggtcg aggtcacggc gtacattccc ggcgagggcc acaacctgca ggagcactcg 240 atggtgctgg tgcgcggcg ccgggtgaag gacctgcctg gtgtgcgcta caagatcatc 300 cgcggttcgc tggatacgca gggtgtcaag aaccgcaaac aggcacgcag ccgttacggc 360 gctaagaagg agaagggctg atgccacgca aggggcccgc gcccaagcgt ccgttggtca 420

<210> 26

<211>21

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> 16S-F amplification primer

<400> 26 ggtgatctgc cctgcacttc g 21 <210>27 <211>21 <212> DNA <213> Mycobacterium tuberculosis <220> <223> 16S-R amplification primer <400> 27 21 cgtcaccca ccaacaagct g <210>28 <211>21 <212> DNA <213> Mycobacterium tuberculosis <220> <223> 16S-5s sequencing primer <400> 28 ggtgatctgc cctgcacttc g 21 <210> 29 <211>21 <212> DNA <213> Mycobacterium tuberculosis <220> <223> 16S-3s sequencing primer <400>29 21 cgtcacccca ccaacaagct g <210>30 <211> 147 <212> DNA <213> Mycobacterium tuberculosis

<220>	
<223> 16S/rrs (streptomycin resistance)	
<400> 30	
cgtgggtgat ctgccctgca cttcgggata agcctgggaa actg	ggtcta ataccggata 60
ggaccacggg atgcatgtct tgtggtggaa agcgctttag cggtg	
gcctatcagc ttgttggtgg ggtgacg	147
<210>31	
<211>21	
<212> DNA	
<213> Mycobacterium tuberculosis	
<220>	
<223> embB-F amplification primer	
<400>31	
eggeaagetg gegeacette a	21
<210> 32	
<211>21	
<212> DNA	
<213> Mycobacterium tuberculosis	
.000	
<220>	
<223> embB-R amplification primer	
<400> 32	
	21
agecageaca etagecegge g	21
<210> 33	
<211>21	
<212> DNA	
<213> Mycobacterium tuberculosis	
215 1.1,000 determine the oriented is	
<220>	
<223> embB-5s seugencing primer	

<400> 33

cggcaagctg gcgcaccttc a

```
<212> DNA
<213> Mycobacterium tuberculosis
<220>
<223> embB-3s sequencing primer
<400> 34
agccagcaca ctagcccggc g
                                               21
<210>35
<211>300
<212> DNA
<213> Mycobacterium tuberculosis
<220>
<223> embB (ethambutol resistance)
<400> 35
eggeatgege eggetgatte eggeaagetg gegeacette accetgaceg aegeegtggt 60
gatattegge tteetgetet ggeatgteat eggegegaat tegteggaeg aeggetaeat 120
cctgggcatg gcccgagtcg ccgaccacgc cggctacatg tccaactatt tccgctggtt 180
cggcagcccg gaggatccct tcggctggta ttacaacctg ctggcgctga tgacccatgt 240
cagegaegee agtetgtgga tgegeetgee agaeetggee geegggetag tgtgetgget 300
<210> 36
<211> 20
<212> DNA
<213> Mycobacterium tuberculosis
<220>
<223> pncA-F amplification primer
<400> 36
atgcgggcgt tgatcatcgt
                                             20
<210>37
<211>20
<212> DNA
<213> Mycobacterium tuberculosis
```

<210> 34 <211> 21

<220>

<223> pncA-F amplification primer

<400> 37

tcaggagctg caaaccaact

20

<210>38

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> pncA-5s sequencing primer

<400>38

atgcgggcgt tgatcatcgt

20

<210>39

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> pncA-3s sequencing primer

<400>39

tcaggagctg caaaccaact

20

<210>40

<211>561

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> pncA (pyrazinamide resistance)

<400> 40

atgegggegt tgatcategt egacgtgeag aacgaettet gegagggtgg etegetggeg 60 gtaaceggtg gegeegget ggeeeggee ateagegaet acetggeega ageggeggae 120 taccateaeg tegtggeaae eaaggaette eacategaee egggtgaeea etteteegge 180 acaceggaet atteetegte gtggeeaeeg eattgegtea geggtaetee eggegeggae 240 tteeateeea gtetggaeae gteggeaate gaggeggtgt tetacaaggg tgeetaeaee 300

ggagcgtaca gcggcttcga aggagtcgac gagaacggca cgccactgct gaattggctg 360 cggcaacgcg gcgtcgatga ggtcgatgtg gtcggtattg ccaccgatca ttgtgtgcgc 420 cagacggccg aggacgcggt acgcaatggc ttggccacca gggtgctggt ggacctgaca 480 gcgggtgtgt cggccgatac caccgtcgcc gcgctggagg agatgcgcac cgccagcgtc 540 gagttggttt gcagctcctg a 561

- <210>41
- <211>20
- <212> DNA
- <213> Mycobacterium tuberculosis
- <220>
- <223> gyrA-F amplification primer
- <400>41

cagctacatc gactatgcga

20

- <210>42
- <211>20
- <212> DNA
- <213> Mycobacterium tuberculosis
- <220>
- <223> gyrA-R amplification primer
- <400>42

gggcttcggt gtacctcatc

20

- <210>43
- <211>20
- <212> DNA
- <213> Mycobacterium tuberculosis
- <220>
- <223> gyrA-5s sequencing primer
- <400> 43

cagctacatc gactatgcga

20

- <210>44
- <211>20

<212> DNA <213> Mycobacterium tuberculosis <220> <223> gyrA-3s sequencing primer

<400> 44

gggcttcggt gtacctcatc

20

<210>45

<211>420

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> gyrA (fluoroquinilone/ciprofloxacin resistance)

<400>45

cgaccggatc gaaccggttg acatcgagca ggagatgcag cgcagctaca tcgactatgc 60 gatgagcgtg atcgtcggc gegcgctgcc ggaggtgcgc gacgggctca agcccgtgca 120 tcgccgggtg ctctatgcaa tgttcgattc eggcttccgc ccggaccgca gccacgccaa 180 gtcggcccgg tcggttgccg agaccatggg caactaccac ccgcacggcg acgcgtcgat 240 ctacgacagc ctggtgcgca tggcccagcc ctggtcgctg cgctacccgc tggtggacgg 300 ccagggcaac ttcggctcgc caggcaatga cccaccggcg gcgatgaggt acaccgaagc 360 ccggctgacc ccgttggcga tggagatgct gagggaaatc gacgaggaga cagtcgattt 420

<210>46

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> 23S-F amplification primer

<400>46

cgaaattcct tgtcgggtaa

20

<210>47

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> 23S-R amplification primer

<400>47

gtatttcaac aacgactcca

20

<210>48

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> 23S-5s sequencing primer

<400>48

cgaaattcct tgtcgggtaa

20

<210>49

<211>20

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> 23S-3s sequencing primer

<400>49

gtatttcaac aacgactcca

20

<210> 50

<211>300

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> 23S (macrolide/azithromycin resistance)

<400> 50

gccccagtaa acggcggtgg taactataac catcctaagg tagcgaaatt ccttgtcggg 60 taagttccga cctgcacgaa tggcgtaacg acttcccaac tgtctcaacc atagactcgg 120 cgaaattgca ctacgagtaa agatgctcgt tacggcggc aggacgaaaa gaccccggga 180 ccttcactac aacttggtat tggtgttcgg tacggtttgt gtaggatagg tgggagactt 240 tgaagcacag acgccagttt gtgtggagtc gttgttgaaa taccactctg atcgtattgg 300